Effect of the Biodegradable Coatings the Base on Microalgae and Oil of the Seed of the Pomegranate in the Conservation Post-Harvest of the Papaya 'Golden'

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Abstract

It is very challenging to the quality of the papaya culture's fruits (*Carica papaya* L.) after the crop, especially due to their significance to the international market, that is, their elevated exportation demands. The purpose of this work was the application of biodegradable coatings composed of *Scenedesmus* sp. and *Chlorella* sp. associated or not with pomegranate seed oil in 'Golden' papaya and to evaluate their effect in the conservation post-harvest. The installation of the experiment was carried out in a completely randomized design, with a 6 x 6 factorial outline, that is, six concentrations (C: control; SO: 0.5% of *Scenedesmus* sp. + 0.3% of pomegranate seed oil; S: 0.5% of *Scenedesmus* sp.; CO: 0.5% of *Chlorella* sp. + 0.3% of pomegranate seed oil; CH: 0.5% of *Chlorella* sp.; O: 0.3% of pomegranate seed oil) and six evaluation periods (0, 3, 6, 9, 12, and 15 days), stored at a temperature of 18 ± 2 °C with $60\pm5\%$ RH with three repetitions of two fruits per portion. The use of coverings composed of *Scenedesmus* sp. and *Chlorella* sp. in association or not with pomegranate seed oil was proven efficient in the reduction of the breathing tax of 'Golden' papaya, delaying the ripening process, and therefore representing a promising alternative for these fruits' post-harvest conservation. The coating composed of 0.5% of *Chlorella* sp. + 0.3% of pomegranate seed oil (CO) provided a better post-harvest conservation of 'Golden' papaya during 15 days of storage.

Keywords: Scenedesmus sp., Chlorella sp., Carica papaya L., quality

1. Introduction

Papaya (*Carica papaya* L.) is a fruit of great economic importance all over the world. It is consumed *in natura* in several countries (Lorenzi et al., 2015). Its main producing countries are India, Brazil, Indonesia, Nigeria, and Mexico. Brazil, the second greatest producer, represents 12.6% of the world production (FAOSTAT, 2015). The papaya tree's fruit is produced in most of Brazil's territory especially in the Northeastern region from which Bahia and Espírito Santo are the best producing states, representing 70% of the Brazilian production (IBGE, 2016). They are cultivated almost for the whole year and their commercialization perspectives for consumption *in natura* in the internal market or for exportation are quite favorable, establishing the culture as one of the most promising fruits for exploration.

However, Brazil is considered as one of the top ten food-wasting countries in the world, with virtually 30% of the production thrown away during the post-harvest phase (Fernandes et al., 2016). In spite of this culture's importance and of the all necessary care during the cultivation phases performed in the production chain, the fruits' commercialization has some problems related to damages caused during the post-harvest (Catanho et al., 2017). The main fruit loss causes are mechanical, or caused by cold, post-harvest diseases, and advanced ripeness (Bautista-Baños et al., 2013). If those wastes were minimized, the papaya fruit farmers and exporters could achieve maximum profits and seize a larger portion of the world market (Ali et al., 2011). Therefore, we can state that one of the Brazilian horticulture's current great challenges is the postharvest fruit quality preservation (Botelho et al., 2016). Consequently, alternative methods are necessary to extend the post-harvest storage of papaya during chain logistics.

A technique that is becoming more popular as a post-harvest treatment for fruits, including the papaya, is the application of biodegradable coatings (Hamzah et al., 2013). Biodegradable coatings are used as an alternative for fruit conservation due to their capacity of changing the atmosphere, reducing the breathing, perspiration and humidity indexes, maintaining pulp firmness, and delaying fruit senescence (Mannozzi et al., 2017). Edible and biodegradable coatings have a great potential to act as transporters of several active ingredients, including antimicrobial agents (Fai et al., 2016; Yousuf & Srivastava, 2017).

Among the several new compounds studied, the pomegranate seed oil, due to its nutritional and medicinal properties, was highlighted as a potential ingredient in the food industry with beneficial attributes to health (Verardo et al., 2014). Its biological potential also makes it an attractive nutraceutical ingredient as a consequence of innovation. It is available at a low cost and is widely accepted by the consumers (Caligiani et al., 2010).

Researches for the development of new products, starting from microalgae, have been proven increasingly promising, since they are sources of natural products that were recently studied for biotechnological applications (Dantas, 2013; Dantas et al., 2015). *Scenedesmus* is a green alga with great potential for industrial applications (Koller et al., 2017; Abdulsamad & Varghese, 2017). It contains all of the essential amino acids and good amounts of proteins, lipids, and macro and microelements (Cheban et al., 2015). *Chlorella* is a unicellular green alga that contains several valuable proteins (40-60%) and that has been thoroughly used in aquaculture, food, and biotechnology industries (Dantas et al., 2015). Microalgae have excellent coating properties or biofilms. Therefore, they are a promising coating or film-forming material due to their rheological and functional characteristics. In the last years, the efficiency of elaborated coatings was evaluated, starting with microalgae in the fruit application (Onias et al., 2016; Queiroga et al., 2017; Oliveira et al., 2018a, 2018b). In this context, this work's objective was to evaluate the application of biodegradable coatings of the *Scenedesmus* sp. and *Chlorella* sp. algae in association or not with pomegranate seed oil for the conservation of post-harvest 'Golden' papaya.

2. Material and Methods

The experiment was developed in the Federal University of Campina Grande (UFCG), Agri-food Science and Technology Center (CCTA), in the Fruit and Vegetable Post-harvest Laboratory. The fruits employed in the experiment were acquired from an orchard located in the meadows of Mamanguape, Paraíba (PB), 385 km from the municipal district of Pombal-PB, in which only the 'Golden' variety was cultivated.

The crop was performed at the morning. The fruits were pre-selected in the field, avoiding the ones with disease symptoms, pathogen presence, or mechanical damage. The fruits' maturation was visually determined. To standardize the experimental sample, we selected the fruits with the following characteristics: fruits with color change, whose yellow spots do not cover more than 15% of their peel (Teodosio, 2014). Afterwards, they were conditioned in a single layer, in cardboard boxes (with the dimensions of 640×480 cm), previously covered with bubble plastic to minimize the impact and friction between them, and taken into the laboratory, where the fruits were selected according to their size uniformity and color. Those with defects or apparent damages caused by the transport were discarded. Later, they were washed with a 1% detergent solution and sanitized with a sodium hypochlorite solution to 200 ppm of active chlorine for 20 minutes. They were then rinsed with water and air dried. The experiment was set up in a completely randomized design, with a 6×6 factorial outline, which comprised six coating techniques (C = control; SO = 0.5% of *Scenedesmus* sp. + 0.3% of pomegranate seed oil; S = 0.5% of *Scenedesmus* sp.; CO = 0.5% of *Chlorella* sp.; O = 0.3% of pomegranate seed oil) and six evaluation periods (0, 3, 6, 9, 12, and 15 days), with three repetitions of two fruits per portion.

The microalgae employed in this study were produced according to Lima (2016), in organic production tanks, at Tamanduá Farm, located in the city of Patos-Paraiba. Once the biomasses were obtained, they were diluted into 2 L of water at constant stirring until the complete homogenization of the solution. Afterwards, the fruits were plunged in the solutions for 20 minutes, after which they were dried at room temperature, conditioned at a bench, and stored at the temperature of 18 ± 2 °C and $60\pm5\%$ RH for subsequent evaluation. For each evaluation period, the fruits were processed in centrifuges and their following features were evaluated:

Loss of Fresh Mass (PMF): determined by a gravimeter at a semi-scale analytical precision of ± 0.01 g. The results were expressed in percentage losses, employing the ratio before the mass loss and after each storage period.

Firmness of the Pulp: evaluated by a texturometer (Fruit Hardness Tester), with a penetration depth of 2.0 mm, speed of 2.0 mm s⁻¹ and a TA 8/1000 ferrule. The readings were made with the whole fruits, and each fruit was measured four times: on their opposite faces and after the removal of portions of their peel. The results were expressed in Newtons (N).

Color of the Peel and Pulp: checked through the digital colorimeter Konica Minolta CR-400 and the CIELAB System, which sets a three-dimensional chromatic space with 3 axes in rectangular coordinates $(L^*a^*b^*)$, which respectively indicate the brightness (L^*) , the red (*a positive) to green (-a* negative) tones, and the yellow (b* positive) to blue (-b* negative) tones. It also sets the cylindrical coordinates (L^*, C^*, H°) . The values of a* and b* are converted into Hue angle (H°) , which represents the color intensity, and their chromes (C^*) , that is, the color purity, according to the equations of (Pinheiro, 2009).

Potential of Hydrogen (pH): measured by a digital peg for the direct reading of the homogenized pulp, according to IAL (2008).

Total Titratable Acidity (ATT): measured according to the methodology recommended by the Adolfo Lutz Institute (2008), using 10 grams of homogenized pulp diluted into 100 mL of distilled water, followed by titration with standardized solution of NaOH 0.1N, using the phenolphthalein turning point as indicator. The results were expressed in citric acid g by 100 g-1 of the sample.

Soluble Solids-(SS): measured directly in the homogenized pulp through a digital refractometer (model PR-100, Palette, Atago Co., LTD., Japan), whose results were expressed in °Brix (AOAC, 2006).

SS/ATT Ratio: calculation of the ratio between soluble solids and total titratable acidity (SS/ATT). The results were expressed in decimals.

Vitamin C: it was measured through Tillman's methods. We weighed 1 g of the sample, which was transferred into Erlenmeyer vials, in which the volume was completed for 50 mL with 0.5% of oxalic acid, whose titration was performed with Tillman's solution until the turning point (AOAC, 2006).

Total Soluble Sugars: The total soluble sugars were measured using Antrona's method (Yemn & Willis, 1954), through a spectrophotometer analysis at 620 nm, and the results were expressed in g 100 g⁻¹ of the pulp.

Total of Carotenoids: measured according to Linchtentháler's methodology (1987). We employed the wavelengths of 470, 646, and 663 nm in a spectrophotometer to perform the reading to determine the carotenoid and chlorophyll concentrations used in a and b of the sample. The results were expressed in g 100 g⁻¹ of the pulp.

Statistical Analysis: The data were submitted for analysis of variance and regression through the Sisvar Program (Ferreira, 2011).

3. Results and Discussion

Starting with the analysis of variance presented in Table 1, we can notice that there was significant interaction between the factors under study (coating and storage time) for the parameters of mass loss, firmness, and peel color (brightness, chromaticity, and hue angle), while the colorimetric parameters of the pulp (brightness, chromaticity, and hue angle) directly proportional to the storage time.

FV	Medium square								
	GL	Mass loss	Firmness	L* pulp	H° pulp	C* pulp	L* peel	H° peel	C* peel
Coating (R)	5	3.65**	2723.73**	9.88 ^{ns}	0.0001 ^{ns}	3.91 ^{ns}	133.86**	0.05**	124.78**
Time (T)	5	230.77**	41403.17**	124.94**	0.06**	146.89**	804.50**	0.52**	1544.45**
$\mathbf{R} imes \mathbf{T}$	25	0.39**	482.14**	2.68 ^{ns}	0.0006 ^{ns}	0.92 ^{ns}	12.02**	0.009**	17.30**
Resídue	72	0.14	8.67	7.37	0.001	4.10	4.14	0.003	7.12
CV (%)	-	8.60	4.7	4.67	0.03	4.79	3.99	0.07	6.95

Table 1. Summary of the analysis of variance for mass loss, firmness, pulp and peel color of 'Golden' papaya with and without the application of biodegradable coatings stored at 18 ± 2 °C with $60\pm5\%$ RH

Note. ns: not significant; ** significant at 1%; * significant at 5%.

The loss of fresh mass was directly proportional to the storage in all coatings types (Figure 1A). Fruits without coating (C) presented a greater mass loss (10.59%), while the coated ones (CO) had a smallest loss of fresh mass (7.84%) in comparison with the other coatings: O: 9.24%, CH: 9.52%, SO: 8.76%, and S: 9.63%. Studies related to the use of microalga-based (*Scenedesmus* sp. and *Chlorella* sp.) biodegradable coatings associated with pomegranate seed oil with the objective of reducing the breathing process of papaya fruits were not found in the literature. Pego et al. (2015), while evaluating the effect of different concentrations of cassava starch used as eatable covering of 'Sunrise soil' papaya, also obtained a directly proportional behavior, presenting an average

of 5% of loss in the last day of the evaluation. Nunes et al. (2017), and Onias et al. (2016), while respectively evaluating papaya fruits and mango, reported that the biodegradable coatings increased the mass loss increased in every treatment during the storage period. However, fruits treated with cassava starch and *Spirulina platensis* obtained lower fresh mass loss values during storage, indicating that the microalga-based coatings protected the fruit against the excessive loss of water into the atmosphere.



Figure 1. Loss of pulp (1A) and firmness (1B) of 'Golden' papaya after the application of biodegradable coatings and over the storage periods, UFCG, Pombal, 2018

The fruits' pulp firmness was inversely proportional to the fruits' coating independent storage period. All coatings were adjusted to linear equations, with the minimum of CO 4.9 N, CH 1.3 N, SO 6.7 N, and S 5.2 N for the 15 days of storage, while the treatments C and O presented values of 0 N for same period. According to Nunes et al. (2017), during the storage period, as the fruits got ripe, there was a change in the fruits' consistence, which is related to the carbohydrates metabolism and to changes in the cell wall, presenting pectin degradation, which causes cell loss and results in the lack tissue firmness of the fruit (Oliveira, 2010).

As seen in Figures 2A, 2B, and 2C, there is a significant increase of the color parameters, with an interaction between the coatings and the storage time in relation to the peel color. According to Motta et al. (2015), this behavior that demonstrates the instrumental reading of the peel color can be used to determine the ripeness stage of papaya fruits. For peel brightness (L*) (Figure 2A), a growing lineal behavior is seen until the last evaluation period, reaching maximums of 59.30, 58.02, 58.26, 57.94, and 57.94 for the O, CO, CH, SO, and S coatings, which had darker fruits in comparison with the control treatment (C-68.45). As for the pulp chromaticity data presented in Figure 2B, an ascending lineal behavior was also verified with maximums of 51.54, 49.94, 47.92, 48.76, 46.13, and 59.70 for the O, CO, CH, SO, S, and C coatings during 15 days of storage. Therefore, the coated fruits delayed their metabolism, while the control treatment presented clearer fruits, indicating a precocious ripening and, consequently, a yellow coloration. As for the color intensity (H°) of the peel, it presented a decreasing lineal behavior in all coatings, reaching minimums of O-90.14, CO-90.18, CH-90.19, SO-90.13, and S-90.09, while the control treatment (C-89.97) presented a more accentuated reduction, in which the color of the fruits developed more quickly from green to yellow, proving that the coatings were more efficient to delay the ripening of the fruits (Figure 2C). Souza et al. (2014), evaluating the ripening physiology of 'Golden' papaya, reported a gradual and uneven change of green to yellow, beginning with yellow stripes and presenting chlorophyll degradation and the synthesis of pigments over the ripening process.

The color of the pulp is one of the main visual features of 'Golden' papaya. It should change from rose-salmon to red-orange during its storage. However the fruits' pulp coloration was not affected by the treatments during storage. Therefore, Figure 2 D, E and F meet the behavior of the color parameters. The coordinate L* (Figure 2D) varies from black (0) to white (100). Therefore, we can notice that the treatments went from a clear coloration to a darker one over time. As for the Chromes (C*), we can notice that, over time, the color went from more intense to red-orange (Figure 2E). All treatments present values under H° to 90°, confirming that the color of the pulp is between yellow and red, being closer to orange. If the Hue angle is between 0° and 90°, the higher it goes, the yellower is the fruit's pulp, and the lower it goes, the redder it is (Mendes et al., 2015). In this study, the decrease of the L* and H° values of the pulp color can be related to the reduction of the total carotenoid content, as seen in Figure 4C.



Figure 2. Coloration of the pulp and peel, L* brightness (2A), Chrome C* (2B), and Hue Angle H° of the peel (2C); L* brightness (2D), Chrome C* (2E), and Hue Angle (H°) of the pulp (2F) of 'Golden' papayas after the application of biodegradable coatings and over the storage periods, UFCG, Pombal, 2018

Table 2 shows that there was a significant interaction between the different coatings and time of storage on titratable acidity, SS/ATT ratio, and vitamin C at the level of 1% of probability for the F test. There was isolated effect of the coating factor only for the carotenoid level and of the time factor for pH, soluble solids, and carotenoids.

FV		Medium square							
	GL	pН	Titratable acidity	Soluble solids	Relation SS/ATT	Vitamin C	Carotenoids	Total sugars	
Coatings (R)	5	0.18 ^{ns}	0.02**	0.24 ^{ns}	29.65**	217.26**	2032.88**	0.003 ^{ns}	
Time (T)	5	1.34**	0.05**	6.61**	51.18**	1552.07**	7652.06**	0.040**	
$\mathbf{R} imes \mathbf{T}$	25	0.09 ^{ns}	0.01**	0.43 ^{ns}	13.56**	68.95**	328.46 ^{ns}	0.001 ^{ns}	
Resídue	72	0.10	0.003	0.34	4.67	4.63	313.08	0.002	
CV (%)	-	6.24	9.15	4.87	10.33	2.61	10.66	12.00	

Table 2. Summary of the analysis of variance for pH, total titratable acidity, soluble solids, SS/ATT ratio, vitamin C, carotenoids, and total sugar of 'Golden' papaya with and without the application of biodegradable coatings stored at 18 ± 2 °C with $60\pm5\%$ RH

Note. ns: not significant; ** significant at 1%; * significant at 5%.

As for the pH, there was not a significant interaction between the evaluated factors (coating \times storage period). However, there was significance for the isolated factor (time of storage), in which there was oscillation of the mean pH values of all samples, increasing during the first days of storage, decreasing from the 3rd day (5.74) to the 12th day (5.04), and increasing again (5.43), as seen in Figure 3A. According to Barragán-Iglesias et al. (2018), the pH level increase happens due to a decrease in the amount of hydrogen ions supplied by organic acids during the ripening process. We can observe that the CO treatment had its lower pH value at its 15th day of storage; there was a tiny decrease of acidity, reflecting its delayed maturation. This pH decrease happened due to the formation of organic acids and sugar in association to the fruits' breathing. Those pH variations over the storage can be attributed to the initial degradation and the subsequent synthesis of organic acids with different potentials of ionic dissociation (Almeida et al., 2006). A similar behavior was reported by Soares et al. (2015), who obtained a pH of 5.85 in 'Golden' fruits using eatable coatings.

The values of the titratable total acidity (ATT) variable are displayed in Figure 3B, which present significant differences in the interaction between the coatings and the storage period. Analyzing the ATT's behavior, we notice that the C treatment kept its ATT values until the 9th day of storage. Afterwards, there was a significant increase of these values to 0.81%. For the CO, SO, and S treatments, there was happened a decrease until the 3rd day, presenting significant increases until the 15th day of 0.71%, 0.73%, and 0.72%. In the O and CH treatments, there was a reduction of acidity up to the 3rd day and, consequently, a significant increase until the 12th day, with reductions until the 15th of 0.39% and 0.57%. We can infer that the treatments under a temperature of 18 °C slowed the papayas' normal ripening process caused by maturation (Pego et al., 2015), while the increase in the acidity in their coatings is probably caused by to the galacturonic acids liberated during the hydrolysis of the cell wall's components, responsible for the tissue firmness (Soares et al., 2015).

Soluble Solids (SS) are considered a quality index in papaya. Their concentration and the composition play an important role in the fruits' flavor, which reflect the maturation phase (Corrêa et al., 2008). The increase of the SS levels contributes to increase the sugar level of the fruit (Nunes et al., 2017). SS have a quadratic behavior and a significant difference during the storage period. At the end of the conservation period, there was an increase of this parameter, for all cases with mean values of 12.15°Brix (Figure 3C). Souza et al. (2014), Teodosio (2014), Mendes et al. (2015), Soares et al. (2015) and Nunes et al. (2017) reported similar behaviors.

The data related to soluble solids and titratable acidity ratio showed that there was significant interaction between the treatments and the time of storage, decreasing from the 3rd day onwards, which corresponds to a quick inhibition of the ripening process. After comparing the data, the final value of the control treatment (15.21) was lower in comparison with the other treatments (O-25.45, CO-17.85, CH-26.13, SO-18.21, and S-16.72), which indicates that the coating grants them a sweeter flavor (Figure 3D). According to Dias et al. (2011), the SS/ATT ratio is an important quality variable in the post-harvest because it expresses the balance between the sweetness and the acidity of the fruit, partly playing an important role in the pleasant sensation in the consumer's palate. Therefore, the greater this value is, the better the level of sweetness will be (Pego et al., 2015). Similar results were reported by Nunes et al. (2017) for 'Formosa' papaya using cassava-starch-based coatings.



Figure 3. PH changes (3A), titratable acidity (3B), soluble solids (3C), and SS/ATT ratio (3D) of 'Golden' papayas after the application of biodegradable coatings and over the storage periods, UFCG, Pombal, 2018

As for total sugars, there was a significant difference in the storage period (Figure 4A): there was a significant increase thereof in all treatments, with an average of 5.16 g 100 g⁻¹ in the 15th day (Figure 3C). The total sugar increases are associated to the soluble solid level increases, although the measurement thereof does not represent the exact sugar level because other substances were also dissolved in the vacuolar sap (vitamins, phenolics, pectins, organic acids, etc.). However, among those, sugars are the most representative element, composing 85-90% of the soluble solids. Oliveira et al. (2018b), evaluating the post-harvest conservation of 'Tommy Atkins' mango with *Chlorella* sp. coating, reported that the sugar totals were not affected with the application of the coatings, indicating that the fruits' flavor did not change.

As for vitamin C, the data were adjusted to cubic equations, presenting variations in the vitamin C content of all tested coatings. The concentration of vitamin C increased up to the 6th day, decreased until the 12th day, and increased again until the 15th day (C-100.10 mg 100 g⁻¹ 83.04 mg 100 g⁻¹, CO-96.16 mg 100 g⁻¹, CH-99.89 mg 100 g⁻¹, SO-93.89 mg 100 g⁻¹, and S-97.64 mg 100 g⁻¹). The C treatment had the greatest vitamin C content until the 15th day of storage but the lowest level thereafter (Figure 4B). The fruit's ascorbic acid level depends on several factors, such as variety, maturation stage, growth means, season, and the acidity (Costa et al., 2010). Nunes et al. (2017), evaluating the 'Formosa' papaya, and Soares et al. (2015), evaluating the 'Golden' papaya, reported similar results in their study of how coatings affect fruits. They reported a gradual increase in this vitamin's level over storage time, which could increase up to 3 times during ripening (Paull & Chen, 1989).

For the carotenoids, significant differences were reported for the factors coating and time of storage separately. We can notice that the greatest means were obtained in the C (178.90 g 100 g⁻¹) and CO (176.89 g 100 g⁻¹) treatments. However, the CH (167.97 g 100 g⁻¹) and S (162.14 g 100 g⁻¹) treatments did not have statistical differences in comparison with the C and CO treatments. Nonetheless, these same treatments were not statistically different from the SO (159.00 g 100 g⁻¹) and O (151.44 g 100 g⁻¹) coatings, which had the lowest means. Since carotenoids are liposoluble compounds, we believe that during the carotenoid synthesis during the storage period, in the SO and O treatments, carotenoids might have migrated to the fruit's peel, thus reacting

with the pomegranate seed oil. For the treatments over the time of storage, we can notice that there was a reduction in the carotenoids level. The papaya fruit has an elevated carotenoid content and a quite pleasant and desirable aroma for consumers. The fruit's pulp color is related to the carotenoid contents and its aroma is considered an important indicator for its commercial value (Waal, 2006; Jing et al., 2015). According to Jing et al. (2015), the carotenoid contents and compositions depend mostly of their cultivation methods and/or of their production areas.



Figure 4. Changes in Total Sugars (4A), Vitamin C (4B), and Carotenoids (4C) of 'Golden' papayas after the application of biodegradable coatings and over the storage periods, UFCG, Pombal, 2018

4. Conclusions

The use of coverings composed of *Scenedesmus* sp. and *Chlorella* sp. in association or not with pomegranate seed oil was proven efficient in the reduction of the breathing tax of 'Golden' papaya, delaying the ripening process, and therefore representing a promising alternative for these fruits' post-harvest conservation.

The coating composed of 0.5% of *Chlorella* sp. + 0.3% of pomegranate seed oil (CO) provided a better post-harvest conservation of 'Golden' papaya during 15 days of storage.

Further studies are recommended to evaluate the effect of eatable microalga-based coverings as a nutritional addictive of several fruits.

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